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論文内容の要旨

The objectives of this study were to isolate and identify halophilic and lactic acid bacteria based on phenotypic, chemotaxonomic, DNA-DNA relatedness and 16S rRNA gene sequencing and to screen and study on diacetyl production of lactic acid bacteria from Thai fermented foods, which is the useful ability for the flavor formation in foods.

Seventeen strains of halophilic lactic acid bacteria were isolated from fermented shrimp paste (*ka-pi*) samples collected at the market in the southern part of Thailand. They were divided into 2 groups. The representative strains in Group I were identified as *Tetragenococcus halophilus* while Group II isolates were identified as *Tetragenococcus muriaticus*. Their phenotypic characteristics, DNA-DNA relatedness and growth on 5% and 10% NaCl were useful for identification of *Tetragenococcus* species. Thirty-two strains of moderately halophilic bacteria were isolated from 6 samples of *Ka-pi* and 3 samples of fish sauce (*nam-pla*) from Thai fish sauce factories at various stages. They were divided in to three groups. Group I (15 strains) were isolated from *Nam-pla*, that were closely related to *Lentibacillus juripiscarius* JCM 12147^T (97.3% similarity) by 16S rRNA gene analysis but they showed low levels of DNA-DNA relatedness (17%) with *L. juripiscarius* JCM 12147^T. Therefore, the strains represent a novel species and the name *Lentibacillus halophilus* sp. nov. is proposed. Two strains (Group II) from *Ka-pi* produced red pigment and non-motile, were closely related to *Lentibacillus salarius* KCTC 3911^T (96.5% similarity based on 16S rRNA sequence). These strains represent a novel species and the name *Lentibacillus kapialis* sp. nov. is proposed. Fifteen strains (Group III) from *Ka-pi* were closely related to *Salinicoccus roseus* JCM 14630^T (97.3 % similarity based on 16S rRNA sequence). They showed low DNA-DNA relatedness to *S. roseus* JCM 14630^T (21.7%). Consequently, these strains represent a novel species and the name *Salinicoccus siamensis* sp. nov. is proposed. Twenty-five of lactic acid bacteria were isolated from fermented tea leaves (*miang*) produced in the northern part of Thailand. They were divided into seven groups. Groups I to VI belonged to *Lactobacillus* and Group VII to *Pediococcus*. Six strains in Group I were identified as *Lactobacillus pantheris*, five strains in Group II as *Lactobacillus pentosus* and four strains in Group V as *Lactobacillus suebicus*. Two strains in Group VI were identified as *Lactobacillus fermentum*. Five strains in Group III are proposed as *Lactobacillus thailandensis* sp. nov., two strains in Group IV are proposed as *Lactobacillus camelliae* sp. nov., and one strain in Group VII is proposed as *Pediococcus siamensis* sp. nov.

Diacetyl-producing lactic acid bacteria screened by using the colorimetric method. The result showed that the most of homofermentative lactic acid bacteria could produce diacetyl higher than the heterofermentative lactic acid bacteria. Seven high producing-diacetyl lactic acid bacteria were selected to study on diacetyl production in MRS broth. The result showed

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all selected strains could produce the maximum of diacetyl concentration at 18 to 36 hours of fermentation time under stationary phase and showed the maximum growth at 12 hours and could produce high diacetyl concentration in the medium containing citrate under static conditions Moreover, diacetyl production production under static condition of strains SR4-2, PM3-14 and AP2-1 could produce high production under static condition of strains SR4-2, PM3-14 and AP2-1 could produce high diacetyl were 3.35, 3.27 and 3.16 mM at pH 5.74, 6.41 and 7.51 after fermentation at 30, 18 and 30 hours respectively. On the basis of the phenotypic and chemotaxonomic characteristics and DNA-DNA hybridization, the strains SR4-2 isolated from soy sauce mash was identified as *Lactobacillus pentosus*, the strain AP2-1 from pork sausage (*mu-yor*) was identified as *Weissella confusa* and the strains PM3-14 (from pasteurized milk) was identified as *Enterococcus faecium*.

As a result, we suggest that we should need continuously isolate from Thai fermented food because various novel LAB species may exist. In addition, we also should decide role of lactic acid bacteria in Thai fermented food.

審査報告概要

本研究はタイで生産される魚醤油，発酵ソーセージ，発酵茶などの発酵食品より乳酸菌や耐塩性菌などの微生物を分離してグルーピングを行い，発酵食品に含まれる菌叢を明らかにするとともに，一部の乳酸菌の生産するジアセチルの生産性について調査したものである。多相分類学的試験の結果，各発酵食品より分離された微生物は 3〜7 グループ分別された。また，その中に 6 種類の新種に相当するグループが含まれていたため，それぞれを新種として提唱し，国際的に認められた。さらに，乳製品などに含まれるジアセチルの生産菌株を取得し，その生産性に関する試験を行った。このように未だ情報の少ないタイの発酵食品に対して多数の菌株の分離を試み，グルーピングすることにより微生物叢を解明したことは学術的価値は高く，また，ジアセチル生産菌の利用の拡大は今後期待されることであり，その生産性や応用利用の可能性について言及したことは重要である。

よって，審査員一同は博士（農芸化学）の学位を授与する価値があると判断した。